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IONIZING RADIATION IN TUMOR PROMOTION AND PROGRESSION

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IONIZING RADIATION IN TUMOR PROMOTION AND PROGRESSION

ABSTRACT

Chronic exposure to beta radiation has been tested as a tumor promoting or progressing agent. The dorsal skins of groups of 25 female SENCAR mice were chemically initiated with a single exposure to DMBA, and chronic exposure to strontium-90/yttrium-90 beta radiation was tested as a stage I, stage II or complete skin tumor promoter. Exposure of initiated mice to 0.5 gray twice a week for 13 weeks produced no papillomas, indicating no action as a complete promoter. Another similar group of animals was chemically promoted through stage I (with TPA) followed by 0.5 gray of beta radiation twice a week for 13 weeks. Again no papillomas developed indicating no action of chronic radiation as a stage II tumor promoter. The same radiation exposure protocol in another DMBA initiated group receiving both stage I and II chemical promotion resulted in a decrease in papilloma frequency, compared to the control group receiving no beta irradiation, indicating a tumor preventing effect of radiation at stage II promotion, probably by killing initiated cells. Chronic beta radiation was tested three different ways as a stage I tumor promoter. When compared to the appropriate control, beta radiation given after initiation as a stage I promoter (0,5 gray twice a week for 13 weeks), after initiation and along with a known stage I chemical promoter (1.0 gray twice a week for 2 weeks), or prior to initiation as a stage I promoter (0.5 gray twice a week for 4 weeks), each time showed a weak (~15% stimulation) bu statistically significant ($p<0.01$) ability to act as a stage I promoter. When tested as a tumor progressing agent delivered to pre-existing papillomas, beta radiation (0,5 gray twice a week for 13 weeks) increased carcinoma frequency from 0,52 to 0,68 carcinoma/animal, but this increase was not statistically significant at the 95% confidence level. We conclude that in the addition to hte known initiating, progressing and complete carcinogenic action of acute exposures to ionizing radiation, chronic exposure to beta radiation can act as a weak stage I tumor promoter of chemically initiated skin cells.

RÉSUMÉ

La présente étude avait pour but d'évaluer le rôle d'expositions répétées de longue durée à des rayonnements bêta comme agent promoteur ou accélérateur de tumeurs cancéreuses. La peau du dos de groupes de 25 souris femelles SENCAR a été initiée chimiquement par une seule exposition à du DMBA, puis exposée pour de longue durées aux rayonnements bêta d'un mélange de strontium 90 et d'yttrium 90 pour juger de leur qualité comme agent suffisant ou comme agent partiel du premier ou du second type de tumeurs de la peau. L'exposition des souris à 0,5 gray deux fois par semaine pendant 13 semaines, n'a produit aucun papillome, ce qui indique que les rayonnements bêta n'ont eu aucun effet comme agent suffisant. Un autre groupe semblable de souris a été initié au TPA pour provoquer le premier type, puis exposé à 0,5 gray de rayonnements bêta deux fois par semaine pendant 13 semaines : là aussi, il n'y a eu aucun papillome, ce qui indique que les rayonnements bêta n'ont eu aucun effet comme promoteur du second type. Lorsque la même procédure a été utilisée sur un autre groupe de souris exposées au DMBA et initiées chimiquement pour provoquer les premier et second types, la fréquence des papillomes a diminué, par rapport au groupe de contrôle non irradié, ce qui indique que les rayonnements bêta semblent avoir un effet inhibiteur sur le développement des tumeurs à partir de cellules du second type, probablement à cause de la mort des cellules exposées. On a ensuite essayé de déterminer de

trois façons différentes comment l'exposition de longue durée aux rayonnements béta pouvait causer le premier type. Comparativement au groupe de contrôle, les rayonnements béta administrés comme promoteur du premier type (0,5 gray deux fois par semaine pendant 13 semaines), ou administrés après la promotion et simultanément à un promoteur chimique du second type (1 gray deux fois par semaine pendant deux semaines) ou encore administrés avant le traitement comme promoteur du premier type (0,5 gray deux fois par semaine pendant quatre semaines), on a pu constater chaque fois une capacité faible (~ 15 % de stimulation), mais significative du point de vue statistique ($p<0,01$), à agir comme promoteur du premier type. Quand on a voulu étudier la capacité des rayonnements béta à accélérer le développement de tumeurs dans des papillomes existants à raison d'une expostion à 0,5 gray deux fois par semaine pendant 13 semaines, on a découvert que le nombre de carcinomes augmentait de 0,52 à 0,68 par animal, mais que cette augmentation n'était pas significative statistiquement à un niveau de probabilité de 95 %. L'étude conclut que, en plus des effets carcinogènes des expositions aiguës aux rayonnements ionisants comme agent promoteur, accélérateur ou suffisant, l'exposition permanente aux rayonnements béta peut agir comme accélérateur faible du premier type dans des cellules cutanées initiées chimiquement.

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INTRODUCTION

1. Background

To date ionizing radiation has been tested only as an agent for cancer initiation, the first step in the multi-step carcinogenesis model. It has been demonstrated to be a skin tumor-initiating agent when irradiated cells were subsequently chronically exposed to a chemical tumor-promoting agent (phorbol ester). The action of chronic radiation in the other two steps of carcinogenesis (promotion and progression) has not been tested, however. The possibility that such ionizing radiation acts as tumor-promoting agent is particularly relevant because of recent evidence implicating oxidizing free radicals as the chemical promoters of tumors. These free radicals are the same DNA-damaging species as are produced by ionizing radiation. This point is also particularly important to the Canadian nuclear industry. Skin is an organ which is constantly in contact with the environment, and human skin undoubtedly contains cells which are "initiated" as a result of DNA damage from chemicals, sunlight or previous ionizing radiation. Atomic Radiation Workers are chronically exposed to lov doses of radiation. If ionizing radiation acts as a tumor-promoting agent, then chronic exposure would stimulate the initiated cells to form tumors. Such a mechanism would also apply to cells elsewhere in the body which may be in the initiated state and could be chronically exposed to radiation. Lung is another organ likely to contain initiated cells resulting from exposure to environmentally generated hydrocarbons, and is also subject to chronic exposure to radon gas. Similar concerns also apply to the possibility that ionizing radiation may act as a progressing agent. Recent evidence has indicated that the risk of a benign tumor progressing to a malignant carcinoma can be substantially increased if the tumor is exposed to DNA damaging chemicals. Most mature humans have a variety of non-malignant skin tumors, most of which have a very lov risk of progression to carcinomas. However, if chronic exposure to ionizing radiation causes sufficient DNA damage, the risk of progression of these tumors may be substantially increased.

The results of this study of ionizing radiation as a tumor promoter or progressor will impact on risk assessments for atomic radiation workers and other workers who are chronically exposed to industrial or medical processes where radiation sources are used.

2. Objectives

To determine whether ionizing radiation, delivered chronically to tissue, acts as a tumor-promoting or -progressing agent for chemically-initiated carcinogenesis.

Abbreviations Used:

MNNG	N-methyl-n'-nitro-N-nitrosoguanidine, a tumor initiator
DMBA	Dimethylbenz(a)anthracene, a tumor initiator
TPA	Tetradecanoylphorbol-13-acetate

1. a complete tumor promoter when applied twice per week for 13 weeks
2. a stage I tumor promoter when applied twice per week for only two weeks

Mezerein	A stage II tumor promoter

METHODS

Live animals (mice) were used to produce skin tumors in a chemically stimulated, initiation, promotion and progression sequence. The test sequence steps were accomplished as follows:

Initiation: a genetic change in a cell which is required before a cell can proceed to become malignant; a heritable mutation.

Groups of 25 female 7-8 week old SENCAR mice were initiated by treatment of their dorsal skins with either 10 nmoles of 7, 12, dimethylbenz(a) anthracene (DMBA) OR 2 μmoles of N-methyl N'-nitro-nitrosoguanidine (MNNG) applied in 200 μl of acetone. Hair was clipped from this area prior to initiation.

Promotion: can be divided into two stages:

Stage I probably involves DNA damage which allows gene expression of the initiating mutation; i.e. probably a further mutation;

Stage II probably involves stimulation of gene expression resulting in an observable phenotype, i.e. production of a visible tumor.

Stage I and II are operationally defined by the sequential application of chemical promoting agents.

In those groups receiving a complete promoter, beginning one week after initiation the initiated area of skin was treated twice per week (Mondays and Thursdays) with 2 μg of tetradecanoylphorbol-13-acetate (TPA) in 100 μl of acetone, for a total of 13-20 weeks.

In those groups receiving stage I promotion (TPA) followed by stage II promotion (mezerein), the TPA treatment was ended after 2 weeks and the mezerein treatment followed immediately (4 μg in 100 μl of acetone, 2x/week, 13 weeks).

Progression: loss of normal gene control, probably the result of a further mutation, such that the tumor becomes malignant; i.e. grows uncontrollably and invasively.

Progression to carcinomas was not stimulated further by chemical means. The influence of radiation was determined by comparing changes in the frequency of progression to carcinomas in groups receiving or not receiving the radiation exposure.

Irradiation

Some groups of animals were irradiated by exposure of the dorsal skin to $^{90}Sr/^{90}Y$ β-rays at a dose rate of 13.4 mGy/sec. The source was covered with a material >100 mg/cm^2 such that ^{90}Sr β-rays were absorbed and the entire exposure was due to ^{90}Y β-rays. Dose rate was observed to decrease linearly with distance into the tissue such that

$$\text{dose rate} = 0.107 - (2.9x)\ (10^{-4})\ \text{Gy sec}^{-1}$$

with x expressed in mg·cm^{-2} for the range 0-140 mg·cm^{-2} at least.

The exposure was limited to an oval area of about 5.75 cm^2 (corresponding to the MNNG or DMBA initiated area) by positioning the animals dorsal side down on a 4 mm thick lead plate with an opening of this area above the source.

All initiation, promotion and irradiation treatments were done with the mice under general anaesthesia from the inhalation anaesthetic methoxyflurane. Treatment and control groups received the same anaesthesia and handling procedures for the same period of time. Where appropriate, control groups received acetone without chemical initiator or promoter. The total number of papillomas per group was recorded twice per week and the average frequency (tumors/animal) calculated from the number of live animals. The average maximum frequency (±1SD) was calculated from the plateau frequencies obtained in the final 6-14 determinations. Carcinoma yield was recorded for 18 months following the beginning of promotion and suspected carcinomas confirmed by histological methods. Carcinoma frequency was calculated from the number of animals alive at the appearance of the first carcinoma.

The data were tested for significance using either the chi-square method or Students t-test for paired samples.

The appearance of papillomas with time is given in figures labelled according to the appropriate "Task". Number of days in the figures refers to days of promotion, or equivalent.

RESULTS

This project was divided into eight separate tasks:

Task 1.
Determine if chronic β-radiation acts as a complete promoting agent.

Test group: The animals were initiated with DMBA and beginning one week later "promoted" with chronic β-radiation (0.5 Gy per fraction twice/week, 13 weeks). No papillomas developed at any time during promotion.

Control group a: This negative control group (DMBA initiation alone, no radiation) also developed no papillomas.

Control group b: The positive control group received DMBA initiation, and beginning one week later, TPA promotion twice per week. This group developed about 10 papillomas/animal.

Carcinomas totalled 13 (in 25 animals, = 0.52) for control group b, 7 (in 23 animals, = 0.30) for control group a and 3 (in 24 animals, = 0.13) for the test group.

These results indicate that chronic β-radiation at this dose rate and over this time period does not act as a complete promoting agent. "Promoting" with chronic β-radiation in DMBA initiated animals apparently decreased the probability that promotion independent initiated cells would progress to carcinomas, from 7/23(=0.30) to 3/24(=0.13) carcinomas/animal but the carcinoma data is not significant (p=0.08) at the 95% confidence level.

Task 2.
Determine if chronic β-radiation acts as a stage I promoter

Test group: DMBA initiated, 0.5 Gy per fraction twice/week for 13 weeks as stage I promotion, followed by stage II promotion (mezerein, 2x/week for 13 weeks). No papillomas existed after stage I (β) promotion (see Task 1 results). After stage II promotion this group developed an average maximum papilloma frequency of 3.99 ± 0.23 per animal.

Control group a: DMBA initiation but no stage I promotion. Initiation was followed one week later by stage II promotion (mezerein, 2x/week, 13 weeks). This group developed an average maximum papilloma frequency of 0.85 ± 0.31 per animal.

Control group b: DMBA initiation, followed by 13 weeks of no treatment (to mimic the period of β-radiation in the test group), followed by stage II promotion with mezerein as in the test group. This group developed an average of 3.46 ± 0.16 papillomas/animal.

These results indicate that there is a natural, time dependent process by which cells move through stage I of promotion (without chemical stimulus) and become available for promotion by a stage II promoter. Chronic β-radiation exposure during this time apparently caused a small increase in the number of cells available for stage II promotion. Based on a comparison of the final 7 observations of average papillomas/animal in the test group versus control group b, the data are significantly different at the 99% confidence level.

Carcinoma frequency was 19/25 animals (= 0.76), in the test group and 17/22(=0.77) in control group b, indicating no difference. Control group a frequency was 9/24(=0.38), significantly different ($p<0.0001$) from control group b. This carcinoma result indicates that chronic β-radiation as a stage I promoter did not detectably increase the number of cells able to progress to a carcinoma.

Task 3.

Determine if chronic β-radiation acts as a stage II promoter

Test group: DMBA initiated, followed 1 week later by stage I promotion (TPA 2x/week, 2 weeks) followed by β-radiation as a stage II promoter (0.5 Gy per fraction, 2x/week, 13 weeks). This group developed an average of 0.04 papillomas per animal.

Control group a: Initiation and stage I promotion as the test group but no radiation. This group developed no papillomas.

Control group b: Initiation and stage I promotion as in the test group, followed by stage II promotion with mezerein (2x/week, 13 weeks). This group developed an average of 3.05 ± 0.15 papillomas/animal and 13 carcinomas on 25 animals (=0.52).

The test group developed 5 carcinomas per 24 animals (=0.21), and control group a developed 4/25(=0.16), not significantly different, indicating no increase in progression probability.

These results indicates that chronic β-radiation does not act as a stage II promoter.

Task 4.

Determine if chronic β-radiation alters the action of a stage I promoter

All groups were initiated with DMBA, received stage I promotion (TPA, 2x/week, 2 weeks) and stage II promotion (mezerein 2x/week, 13 weeks).

Control group: No additional treatment. This group developed an average of 3.47 ± 0.22 papillomas per animal and a total of 19 carcinomas on 24 animals (=0.79).

Test group 1: Received 0.5 Gy per fraction, 2x/week for 2 weeks along with stage I promotion by TPA. This group developed an average of 3.17 ± 0.23 papillomas per animal and a total of 13 carcinomas on 21 animals (=0.62).

Test group 2: As test group 1 except 1.0 Gy. This group developed 3.91 ± 0.17 papillomas per animal and a total of 19 carcinomas on 25 animals (=0.76).

Test group 3: As test group 1 except 2.5 Gy. This group developed an average of 1.45 ± 0.15 papillomas per animal and a total of 9 carcinomas on 24 animals (=0.38).

These results indicate that a higher dose and dose rate of β-radiation (test group 3, 2.5 Gy, 2x/week, 2 weeks), at the time of stage I promotion can reduce the probability of both papillomas ($p<0.001$) and carcinomas ($p<0.0001$). At a lower dose and dose rate however (0.5 Gy, 2x/week, 2 weeks) there was no significant indication that the β-radiation interacted either in a positive or negative way with the chemical (TPA) induced stage I promotion or in the progression of initiated cells to carcinomas. At an intermediate dose and dose rate (1.0 Gy, 2x/week, 2 weeks), the average number of papillomas per animal increased from 3.47 to 3.91. This increase is significant ($p<0.01$). There was no significant change in the probability that initiated cells would progress to carcinomas.

Task 5.
Determine if β-radiation alters the action of a known stage II promoter

Control group: DMBA initiation followed by stage I promotion (TPA, 2x/week, 2 weeks) and then stage II promotion (mezerein, 2x/week, 13 weeks). This group developed an average of 6.05 ± 0.29 papillomas per animal and a total of 13 carcinomas on 24 animals (=0.54).

Test group: As the control group except receiving 0.5 Gy per fraction, 2x/week for 13 weeks along with the stage II promoter mezerein. The group continued to receive mezerein alone for an additional 13 weeks. This group developed an average of 4.79 ± 0.26 papillomas per animal after 13 weeks of mezerein + radiation, and remained about constant for the following 13 weeks of mezerein alone without radiation. A total of 11 carcinomas developed on 24 animals (=0.46).

This data indicates that chronic β-radiation during the time of stage II promotion with mezerein significantly ($p<0.001$) decreased the number of papillomas that appeared. There was no significant effect of β-radiation on the probability of initiated cells progressing to carcinomas.

Task 6.
Determine if chronic β-radiation alters the action of a complete promoter

Control group: DMBA initiated, followed by TPA promotion (2x/week, 15 weeks). This group developed an average of 14.21 ± 0.30 papillomas per animal and a total of 14 carcinomas on 21 animals (=0.67).

Test group: As control group except receiving 0.5 Gy per fraction, twice/week for 15 weeks along with TPA promotion. This group developed an average of 9.88 ± 0.54 papillomas/animal and a total of 12 carcinomas on 22 animals (=0.55).

These results indicate that chronic β-radiation, during the time of exposure to a complete promoter, significantly decreased the probability of papilloma formation ($p<0.001$). The radiation did not significantly alter the probability that an initiated cell would progress to a carcinoma.

Task 7.
Determine if chronic β-radiation delivered to pre-existing tumors will influence progression.

Control group: DMBA initiation followed by TPA promotion for 13 weeks. This group developed an average of 9.72 ± 0.45 papillomas/animal at the end of promotion and a total of 13 carcinomas on 25 animals (=0.52).

Test group: As the control group except received 0.5 Gy per fraction, 2x/week for 13 weeks after the end of TPA promotion. This group developed 9.64 ± 0.44 papillomas/animal at the end of promotion. By the end of the irradiation the average papilloma frequency had declined to 8.57 ± 0.23, a significant change ($p<0.001$). This group developed a total of 15 carcinomas on 22 animals (=0.68).

This result indicates that chronic β-radiation delivered to pre-existing papillomas increased the carcinoma frequency from 0.52 to 0.68 but this increase was not significant at the 95% level of confidence.

Task 8.
Determine if chronic β-radiation prior to initiation will influence tumor progression.

All groups were initiated with MNNG and beginning one week later promoted with the complete promoter TPA (2x/week, for 20 weeks) except as noted.

Test group 1: Received 0.5 Gy per fraction, 2x/week, for 4 weeks prior to initiation. This group developed an average of 5.47 ± 0.37 papillomas per animal and a total of 10 carcinomas on 24 animals (=0.42).

Test group 2: Received 1.0 Gy per fraction, 2x/week for 4 weeks prior to initiation. This group developed an average of 4.00 ± 0.08 papillomas per animal and a total of 12 carcinomas on 25 animals (=0.48).

Control group a: Received no additional treatment. This group developed an average of 4.73 ± 0.23 papillomas/animal and 14 carcinomas on 25 animals (=0.56).

Control group b: Received 0.5 Gy per fraction, 2x/week for 4 weeks, were not initiated with MNNG, but were promoted with TPA (2x/week for 20 weeks). This group developed an average of about 0.4 papillomas per animal after about 16 weeks but this declined to about 0.2 at 20 weeks. This group developed a total of 7 carcinomas on 22 animals (=0.32).

Control group c: Received 0.5 Gy per fraction, 2x/week for 4 weeks prior to MNNG initiation, but then received no TPA promotion. This group developed an average of 0.04 papillomas/animal and a total of 4 carcinomas on 19 animals (=0.21).

Control group d: Received 0.5 Gy per fraction, 2x/week for 4 weeks, followed by neither MNNG initiation nor TPA promotion. This group developed no papillomas and 5 carcinomas on a total of 24 animals (0.21).

These results indicate that a chronic β-radiation exposure (0.5 Gy, 2x/week for 4 weeks) prior to MNNG initiation, followed by promotion with a complete promoter, significantly increased (from 4.85 to 5.57) the number of papillomas that appeared ($p<0.001$). At a higher dose of 1.0 Gy per exposure, the average number of papillomas declined significantly from 4.85 to 4.00 ($p<0.001$).

The carcinoma frequency was not changed by the 1.0 Gy dose fractions before initiation (frequency = 0.48 and 0.56 with or without β-irradiation respectively). The 0.5 Gy dose fractions apparently reduced the carcinoma frequency from 0.56 to 0.42, but this is not significant at the 95% confidence level.

The results also show that insignificant numbers of papillomas developed when TPA promotion was absent.

DISCUSSION

These experiments tested the action of ^{90}Y-β-radiation exposures on various steps in a multi-stage mouse skin tumor formation system. The radiation was delivered in a fractionated (2x/week) dose schedule, spread out over weeks, to approximate a chronic β-radiation exposure to skin. Several specific tests or "tasks" were performed.

The data from Task 1 (Results) indicate quite clearly that chronic β-radiation does not act as a complete tumor promoter. The DMBA initiated animals (receiving 0.5 Gy fractions twice a week for 13 weeks; total dose = 13 Gy) developed no papillomas, compared to the positive control group promoted with the complete promoter TPA, that developed about 10 papillomas/animal. Control, initiated animals receiving no promotion (TPA or radiation) also developed no papillomas.

Task 2 examined the possibility that chronic β-radiation may act as a stage I promoter. The evidence indicates that 0.5 Gy fractions twice a week for 13 weeks (total =13 Gy) produced a 20% increase in papillomas, in initiated animals subsequently receiving stage II promotion. This result was significant at the 99% confidence level and indicates that chronic β-radiation does act as a weak stage I promoter. This conclusion from the Task 2 data is supported by other data in the Task 4 section. In Task 4 we added β-radiation to stage I chemical promotion (TPA, 2x/week, 2 weeks). At the lower 0.5 Gy dose/fraction (total dose = 2.0 Gy) no effect was seen, but at 1.0 Gy/fraction (total dose = 4.0 Gy) a statistically significant 13% increase in tumors occurred. Data from Task 8 suggest that irradiation prior to initiation may also effectively increase stage I promotion. An exposure of 0.5 Gy/fraction for 4 weeks (total dose = 4.0 Gy) prior to initiation increased papilloma numbers 15%. The increase was significant at the 99% confidence level. Since the radiation exposure was delivered prior to initiation, this result suggests that radiation is not acting directly on the initiated cells, but is stimulating a cellular process that causes completion of stage I promotion. The data of Task 2 clearly indicate that such a natural process exists, since allowing an increased time between initiation and stage II chemical promotion (i.e. time for a natural stage I promotion process to occur) increased the papilloma frequency more than 3.5 fold.

Task 3 tested chronic β-radiation for its action as a stage II tumor promoter, and the data from these experiments clearly indicate that there was no significant tumor formation when DMBA initiated, stage I promoted animals subsequently received 0.5 Gy 2x/week for 13 weeks (total dose = 13 Gy), a result not different from the unirradiated control group. In fact, the data in Task 5 suggest that chronic β-radiation can act as a stage II promotion inhibitor since 0.5 Gy, 2x/week for 13 weeks (total dose = 13 Gy) along with chemical stage II promotion (in initiated and chemical stage I promoted animals) decreased papilloma frequency 21% ($p<0.001$). This inhibitory effect is also supported by data from Task 6 where chronic β-radiation (0.5 Gy, 2x/week, 15 weeks, total dose = 15 Gy) given along with a complete promoter (TPA) reduced papilloma frequency by 31% ($p<0.001$). These data indicate therefore that chronic β-radiation inhibits stage II tumor promotion, and

when given with a complete promoter, the inhibitory effect on stage II promotion over-rides the stimulatory effect of radiation on stage I promotion. This result is consistent with the idea of sequential stages of promotion, where stage I promotion must be followed by stage II, and therefore a decrease in the probability of stage II controls the overall risk.

Task 4 was an experiment to determine if chronic β-radiation alters the action of a known stage I promoter. Three different dose fractions were used (0.5, 1.0 and 2.5 Gy) along with chemical (TPA) stage I promotion, 2x/week for 2 weeks. Compared to unirradiated controls, the data indicate no effect at the lowest dose fraction (0.5 Gy; total dose = 2Gy), a stimulation of stage I at 1.0 Gy/fraction (total dose = 4.0 Gy) ($p<0.01$) and an inhibition at the highest dose fraction (2.5 Gy; total dose = 10Gy). As discussed above, the stimulatory effect at the intermediate dose is consistent with other data indicating that chronic β-radiation acts as a weak stage I promoter. At higher doses and dose rates however (10 Gy at 2.5 Gy/fraction) papilloma frequency decreased 58% ($p<0.0001$). This result suggests that high doses delivered in a more acute fashion (over 2 weeks) likely killed some of the initiated cells, preventing any promotional effects. The data suggest therefore that the promotional stage I stimulatory effects of chronic β-radiation are simply added to the effects of chemically stimulated stage I promotion, but that higher dose rates can kill the initiated cells, eliminating any possibility of promotion.

Task 5 examined the action of chronic β-radiation on a stage II tumor promoter and Task 6 its action on a complete promoter. As discussed above, the data indicate an inhibitory effect on stage II promotion, when given either with a specific stage II (chemical) promoter, or a complete (stage I + II) promoter. The papilloma data indicate that this inhibitory effect is due to a loss (killing) of initiated cells, since further stage II promotion with mezerein but without radiation did not increase the papilloma number.

In Task 7, chronic β-radiation was applied to mice already bearing papillomas from an initiation, promotion exposure protocol. This experiment tested the influence of this radiation on the probability that these tumors would progress to carcinomas. The data show that the carcinoma frequency did increase by 31% but this increase was not significant at the 95% confidence level ($p=0.12$). This increase may be real but the increase in the number of carcinomas was insufficient to provide statistical significance. A real increase would be consistent with the effect of acute γ radiation, known to act as a tumor progressing agent in this system. A statistically significant effect of chronic β-radiation therefore would require an increased number of animals.

Task 8 examined the effect of chronic β-radiation, applied prior to tumor initiation (with MNNG), on tumor progression to carcinomas. The initiator MNNG was chosen instead of DMBA because previous data from this and other laboratories indicated that a single acute β-radiation exposure at the time of initiation could stimulate DNA repair and reduce the probability of

tumor formation. The data presented here however indicate that chronic β-radiation applied prior to initiation at either of the dose rates used did not significantly alter the probability of tumor progression.

Data on the influence of chronic radiation on tumor progression is also available from the results of the other "Tasks". Although chronic β-radiation acted as a weak stage I promoter in Task 2, it did not cause a significant increase in progression to carcinomas. However, since the papilloma stimulation was only 20%, a similar increase in carcinomas would not be detectable with the number of animals used.

This lack of influence of chronic β-radiation on progression was also noted in Task 4 where the radiation was given along with the stage I promoter. Carcinoma frequency was not different from the unirradiated control at either the 0.5 or the 1.0 Gy dose/fraction rate. At 2.5 Gy/fraction however, the carcinoma frequency declined significantly (52% decline; $p<0.0001$). This result was consistent with the 58% decline in papilloma frequency noted in this group (discussed above) and reinforces the idea that the decline in due to killing of initiated cells at this relatively high dose and dose rate.

Chronic β-radiation applied either alone as a stage II promoter (Task 3), or in combination with a chemical stage II promoter (Task 5) did not significantly change the probability of progression to carcinomas in either case. This result indicates that chronic β-radiation (at this dose and dose rate, 0.5 Gy/fraction, 2x/week for 13 weeks) does not detectably influence progression of either stage II promotion dependent cells (Task 5) or stage II promotion independent cells (Task 3). It should be noted though, that the radiation produced a significant decrease (21%) in the papilloma frequency in Task 5, and the measured carcinoma frequency also declined 15% even though this was not statistically significant. An actual (but again not statistically significant) decline in carcinoma frequency (18%) was also measured in Task 6 where chronic β-radiation was delivered along with a complete promoter. This can be compared to the significant 31% decline in papilloma frequency in the same group. Although the decline in carcinoma numbers is not large enough to be statistically significant in any of these cases, the trend is consistent and corresponds to the significant decline in papilloma frequencies in each case. This result supports the idea that the observed inhibition of stage II promotion by chronic β-radiation may, in fact, also result in reduced progression to carcinomas. The reduction could be due to either cell killing or to inhibition of the stage II promotion process, but the permanent loss of initiated cells shown in the Task 5 test group, where stage II promotion was continued in the absence of radiation, indicates cell killing may be the mechanism.

Additional statistical tests

One conclusion of this work was that β-radiation acts as a weak stage I promoter. This conclusion arose from the data of three separate sets of experiments, listed in Tasks, 2, 4 and 8. These data were subjected to additional statistical tests for significance. The papilloma frequency measured in the final seven observations of the appropriate control and test groups were evaluated.

Task 2.
Test group and control group b

Students t-test	$p = 5.76 \times 10^{-3}$
One-way analysis of variance	$p = 2.86 \times 10^{-4}$
Two-tailed signed rank test	$p = 0.0156$
Two-tailed Wilcoxon rank sum test	$p = 5.83 \times 10^{-4}$
Spearmans rank correlation coefficient	$= 0.50$

Each test indicates that the two groups are significantly different.

Task 4.
Control group and test group 2

Students t-test	$p = 4.56 \times 10^{-3}$
One-way analysis of variance	$p = 1.35 \times 10^{-3}$
Two-tailed signed rank test	$p = 0.0156$
Two-tailed Wilcoxon rank sum test	$p = 2.33 \times 10^{-3}$
Spearmans rank correlation coefficient	$= 0.14$

Each test indicates that the two groups are significantly different.

Task 8.
Control group a and test group 1

Student t-test	$p = 9.34 \times 10^{-3}$
One-way analysis of variance	$p = 6.73 \times 10^{-4}$
Two-tailed signed rank test	$p = 0.0156$
Two-tailed Wilcoxon rank sum test	$p = 1.17 \times 10^{-3}$
Spearmans rank correlation coefficient	$= 0.46$

Each test indicates that the two groups are significantly different.

CONCLUSIONS

1. Chronic β-radiation acts as a weak stage I promotion agent, probably by stimulating a naturally occurring time-dependent (non-chemically stimulated) stage I promotion.

2. Chronic β-radiation does not act as either a complete or stage II promoting agent.

3. Chronic β-radiation inhibits stage II promotion by a chemical promoter, probably by killing some initiated cells.

4. Evidence that chronic β-radiation acts as a tumor progressing agent is suggestive but not statistically significant.

SOURCES OF ERROR

1. COUNT OF PAPILLOMAS IS VISUAL, CONFIRMED BY FEEL

2. PAPILLOMAS APPEAR AND REGRESS WITH TIME

3. AT LATE TIMES, PAPILLOMAS BECOME LARGE AND MAY GROW TOGETHER

4. APPEARANCE OF A CARCINOMA DURING PROMOTION OBSCURES PAPILLOMAS

6. THERE ARE AT LEAST TWO TYPES OF INITIATED CELLS
   - PROMOTION DEPENDENT
   - PROMOTION INDEPENDENT

RECOMMENDATION

The object of the work presented here was to determine whether ionizing radiation, delivered chronically to tissue, acts as a tumor-promoting or -progressing agent for chemically initiated tumors. Indications of a positive effect were observed when β-radiation was used as a stage I promoter. Three independent experiments, testing β-radiation as a stage I promoter in three different circumstances, all showed a positive effect. While the absolute magnitude of this effect was small, each result was statistically significant and the three independent and different experiments lend weight to the conclusion. It should be noted that the purpose of the experiment was to detect any effect, and as such the methods did not attempt to define the magnitude or limits of radiation as a promoter. It is specifically recommended therefore that

a) Confirmation of the general finding, of β-radiation as a stage I promoter, be sought in additional tests
b) That experiments be designed to explore the effect of various doses and dose rates, to define the boundaries, limits, and optimums for this promotional effect
c) If warranted by the above experiments, radiations of different LET be used to compare promotional efficiencies.

IMPLICATIONS FOR HUMAN EXPOSURES TO CHRONIC RADIATION

This study indicates that a chronic β-radiation exposure acts as a weak stage I promoter, or stimulates the naturally occurring processes of stage I promotion. Whatever the mechanism, the practical consequences are the same: an increase in the probability that previously initiated skin cells will ultimately form a tumor and therefore an increase in tumor frequency. These experiments demonstrated that such an effect exists but did not attempt to define dose rates or total doses at which this process is maximum or minimum, nor did it attempt to define procedures by which this process might be suppressed.

Since this process exists however, it will impinge on any epidemiological estimate of the biological effect of a radiation exposure. Consider for example, two group of individuals, each of whom were exposed to some acute radiation exposure. This will produce initiated cells that will progress to carcinomas with some probability. If however one group is subsequently exposed chronically to low doses of ionizing radiation, this will have the effect of increasing the probability of stage I promotion, and hence of tumor formation. The second group will therefore appear to be at greater risk from the initial radiation dose than the former group.

As a second example consider two groups of atomic radiation workers, equally exposed to chronic low doses of radiation. If however one group had been previously exposed to chemical initiators, either as a result of previous work history or life style, the apparent risk of the low chronic radiation exposure may be <u>much</u> greater in that group.

The data presented indicate that such chronic exposures may increase tumor frequency by relatively small amounts; we observed increases in the 20% range. As noted above however, "optimum" doses and dose rates were not explored and therefore the magnitude of the effect may vary around this number. The limits are unknown except that the data shows higher dose rates may reduce the effect, apparently by killing initiated cells.

Summary Tables

TASK 1

IS CHRONIC β-RADIATION A
COMPLETE PROMOTING AGENT ?

TREATMENT	TUMORS/ANIMAL	CARC/ANIMAL
I	0	0.30
I → 0.5 Gy β (13 wks)	0	0.13 p<0.08 N.S.
I → TPA (13 wks)	10	0.52

CONCLUSION: NO

NOTE: ABOUT HALF OF THE CARCINOMAS RESULT FROM PROMOTION INDEPENDENT INITIATED CELLS. CHRONIC RADIATION MAY KILL THESE CELLS.

TASK 2

IS CHRONIC β-RADIATION A
STAGE I PROMOTER ?

TREATMENT	TUMORS/ANIMAL	CARC/ANIMAL
I → MEZ (13 wks)	0.85 ± 0.31 p<0.001	0.38 p<0.0001
I → NO TREATMENT (13 wks) → MEZ (13 wks)	3.46 ± 0.16 p<0.01	0.77
I → 0.5 Gy β (13 wks) → MEZ (13 wks)	3.99 ± 0.23	0.76

CONCLUSION: YES, WEAK.

TASK 3

IS CHRONIC β-RADIATION A STAGE II PROMOTER ?

TREATMENT	TUMORS/ANIMAL	CARC/ANIMAL
I → TPA (2 wks) → 0.5 Gy β (13 wks)	0.04	0.21
I → TPA (2 wks) → NO TREATMENT (13 wks)	0.0	0.16
I → TPA (2 wks) → MEZ (13 wks)	3.05 ± 0.15	0.52

CONCLUSION: NO

NOTE: CHRONIC RADIATION DID NOT SIGNIFICANTLY ALTER PROGRESSION IN TUMORS PROMOTED TO STAGE I ONLY.

TASK 4

DOES CHRONIC β-RADIATION ALTER THE ACTION OF A STAGE I PROMOTER ?

TREATMENT	TUMORS/ANIMAL	CARC/ANIMAL
I → TPA (2 wks) → MEZ (13 wks)	3.47 ± 0.22	0.79
I → TPA + 0.5 Gy β (2 wks) → MEZ (13 wks)	3.17 ± 0.23 N.S.	0.62
I → TPA + 1.0 Gy β (2 wks) → MEZ (13 wks)	3.91 ± 0.17 $p<0.01$	0.76
I → TPA + 2.5 Gy β (2 wks) → MEZ (13 wks)	1.45 ± 0.15 $p<0.001$	0.38 $p<0.0001$

CONCLUSION: YES, INCREASES (ADDITIVE ?)

BUT HIGHER DOSE RATE KILLS INITIATED CELLS.

TASK 5

DOES CHRONIC β-RADIATION ALTER THE ACTION OF A STAGE II PROMOTER ?

TREATMENT	TUMORS/ANIMAL	CARC/ANIMAL
I → TPA (2 wks) → MEZ (13 wks)	6.05 ± 0.29	0.54
I → TPA (2 wks) → MEZ + 0.5 Gy β (13 wks) ↓ MEZ (13 wks)	4.79 ± 0.26 $p<0.001$	0.46 N.S.

CONCLUSION: YES, INHIBITS, PROBABLY BY KILLING INITIATED CELLS.

TASK 6

DOES CHRONIC β-RADIATION ALTER THE ACTION OF A COMPLETE PROMOTER ?

TREATMENT	TUMORS/ANIMAL	CARC/ANIMAL
I → TPA (15 wks)	14.21 ± 0.30	0.67
I → TPA + 0.5 Gy β (15 wks)	9.88 ± 0.54 $p<0.001$	0.55 N.S.

CONCLUSION: YES, INHIBITS, PROBABLY BY KILLING INITIATED CELLS.

TASK 7

DOES CHRONIC β-IRRADIATION OF PRE-EXISTING TUMORS ALTER PROGRESSION ?

TREATMENT	TUMORS/ANIMAL	CARC/ANIMAL
I → TPA (13 wks)	9.72 ± 0.45	0.52
I → TPA (13 wks) → 0.5 Gy β (13 wks)	9.64 → 8.57	0.68

CONCLUSION: CARCINOMA FREQUENCY INCREASED BY 30% BUT THIS WAS STATISTICALLY NOT SIGNIFICANT.

TASK 8

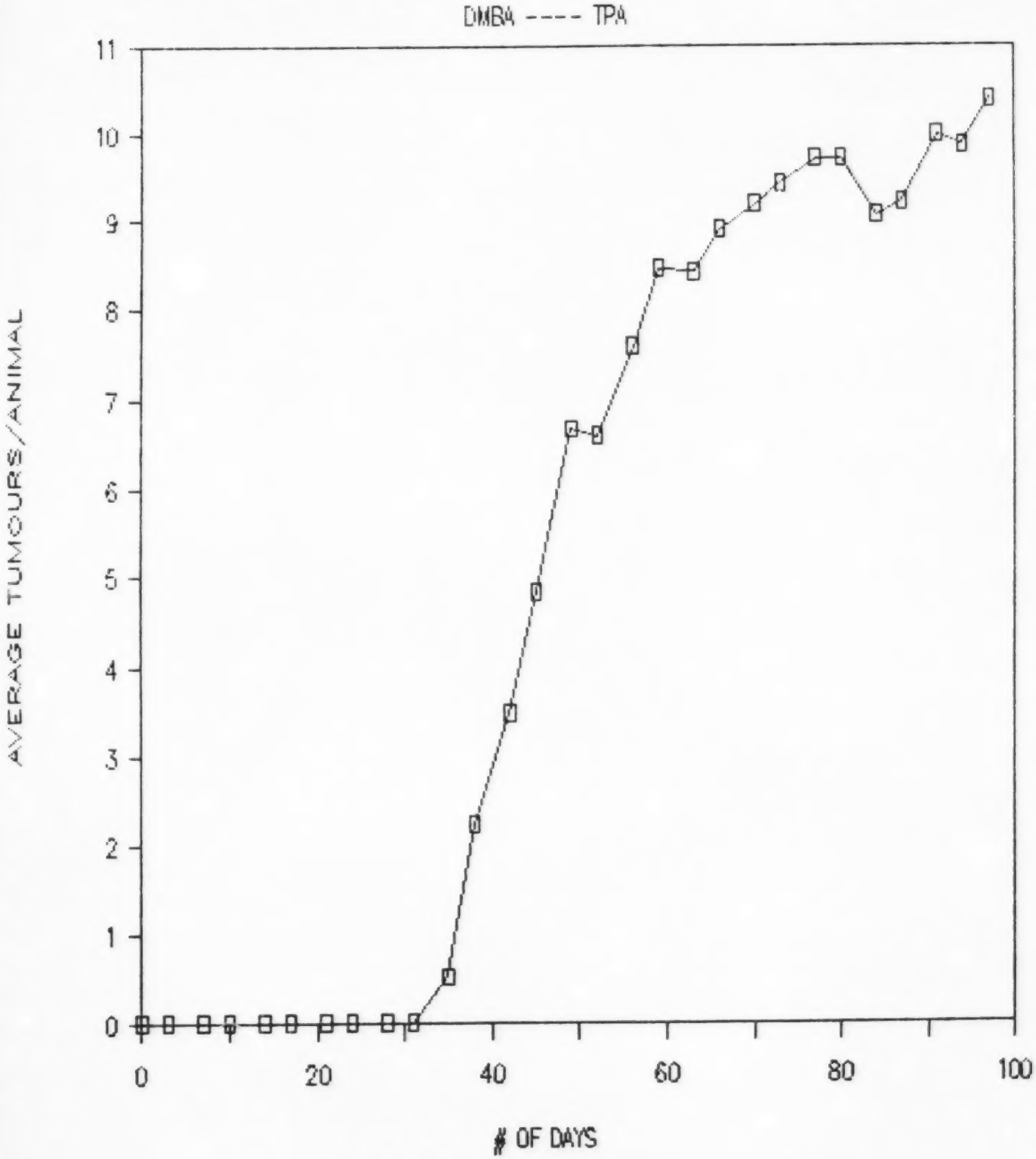
DOES CHRONIC β-IRRADIATION PRIOR TO INITIATION INFLUENCE PROGRESSION ?

TREATMENT	TUMORS/ANIMAL	CARC/ANIMAL
I → TPA (20 wks)	4.73 ± 0.23	0.56
0.5 Gy β (4 wks) → I → TPA (20 wks)	5.47 ± 0.37 p<0.001	0.42
1.0 Gy β (4 wks) → I → TPA (20 wks)	4.00 ± 0.08 p<0.001	0.48
0.5 Gy β (4 wks) → X → TPA (20 wks)	0.40 → 0.20	0.32
0.5 Gy β (4 wks) → I → No Treatment (20 wks)	0.04	0.21
0.5 Gy β (4 wks) → X → No Treatment (20 wks)	0.0	0.21

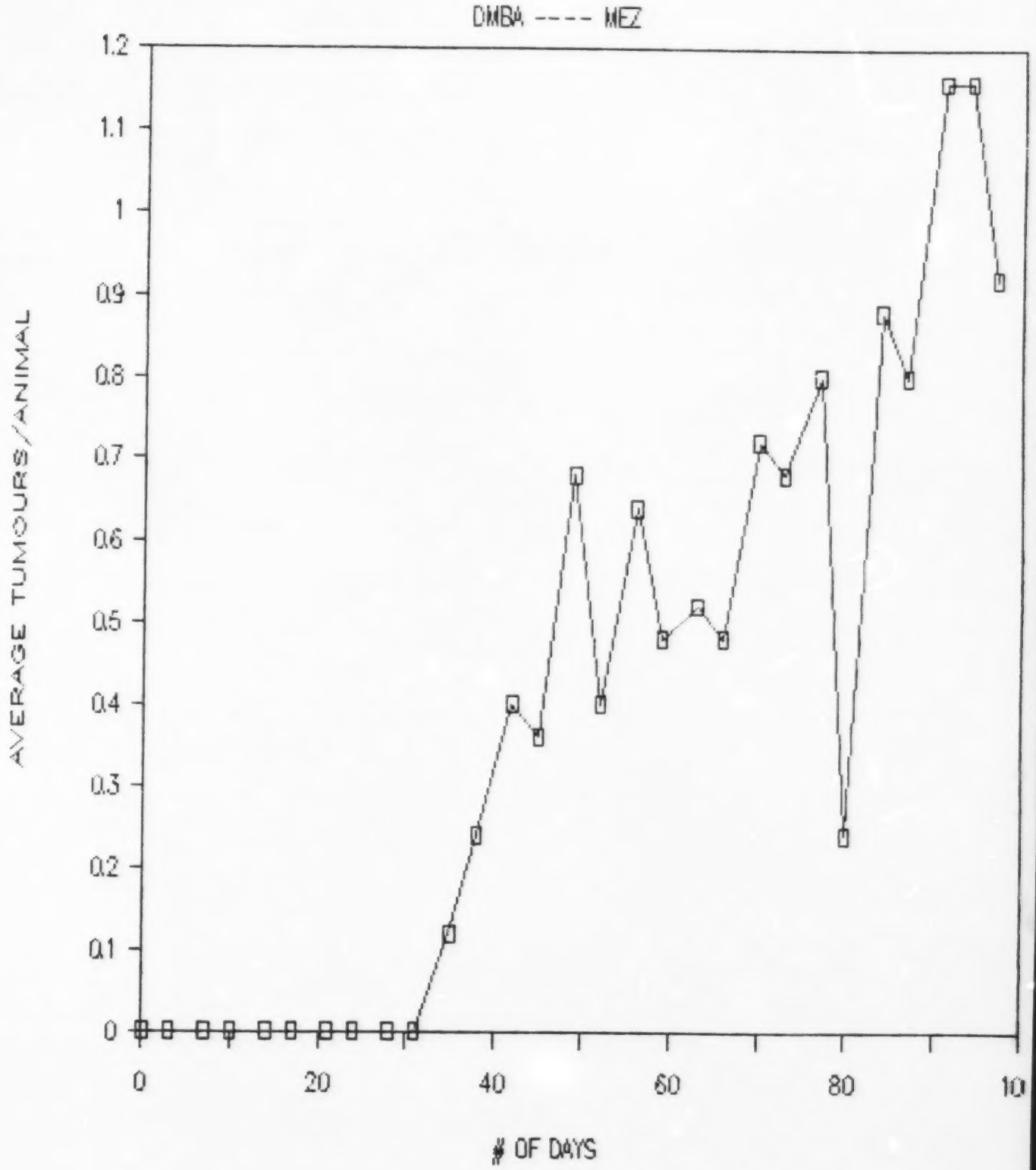
CONCLUSION: NO

NOTE: LOW DOSE RATE β PRIOR TO INITIATION MAY INCREASE TUMOR NUMBER (BY STIMULATING A STAGE I PROMOTION PROCESS ABLE TO ACT ON INITIATED CELL PRODUCED LATER).

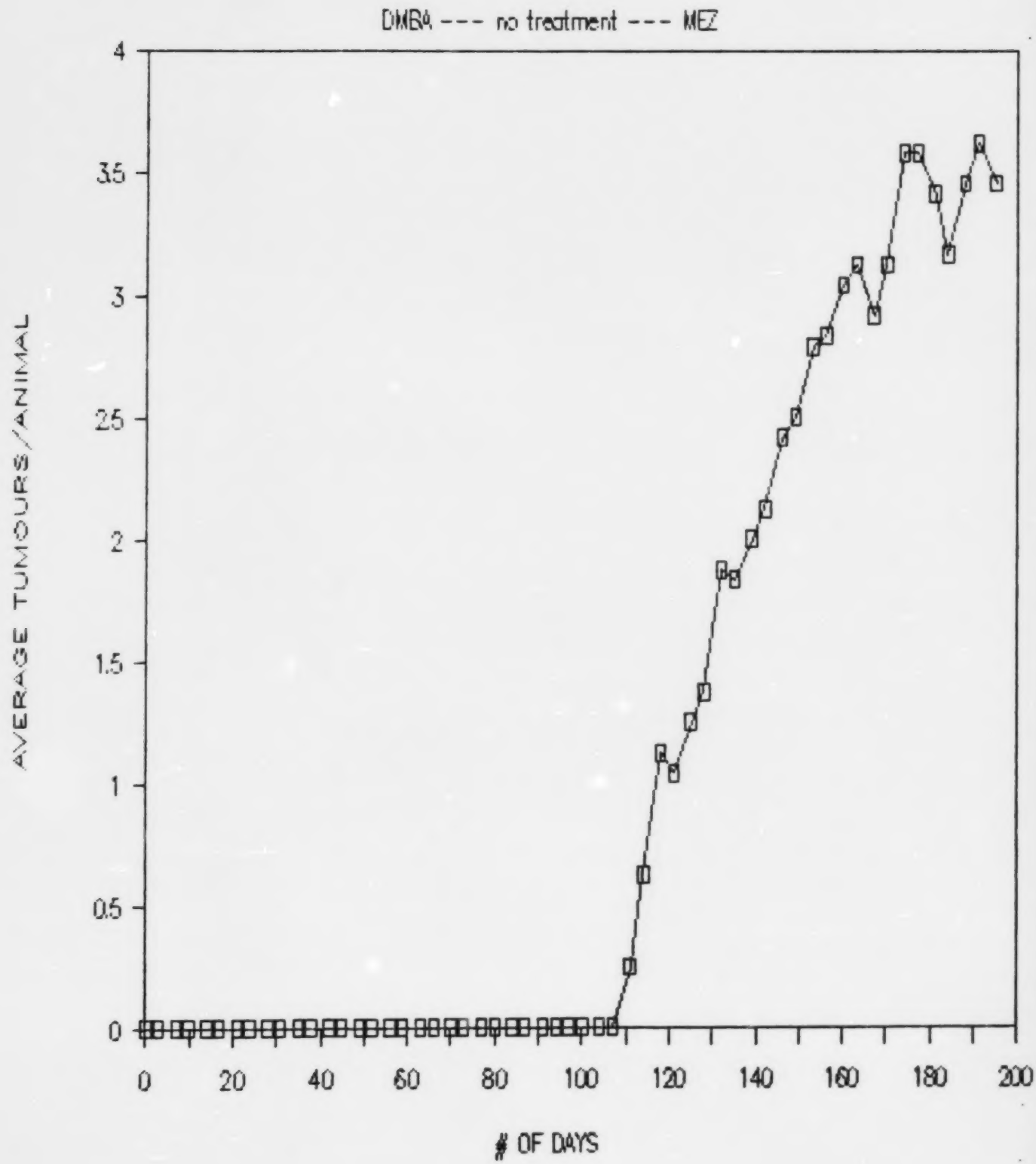
TASK 1 CONTROL GROUP b



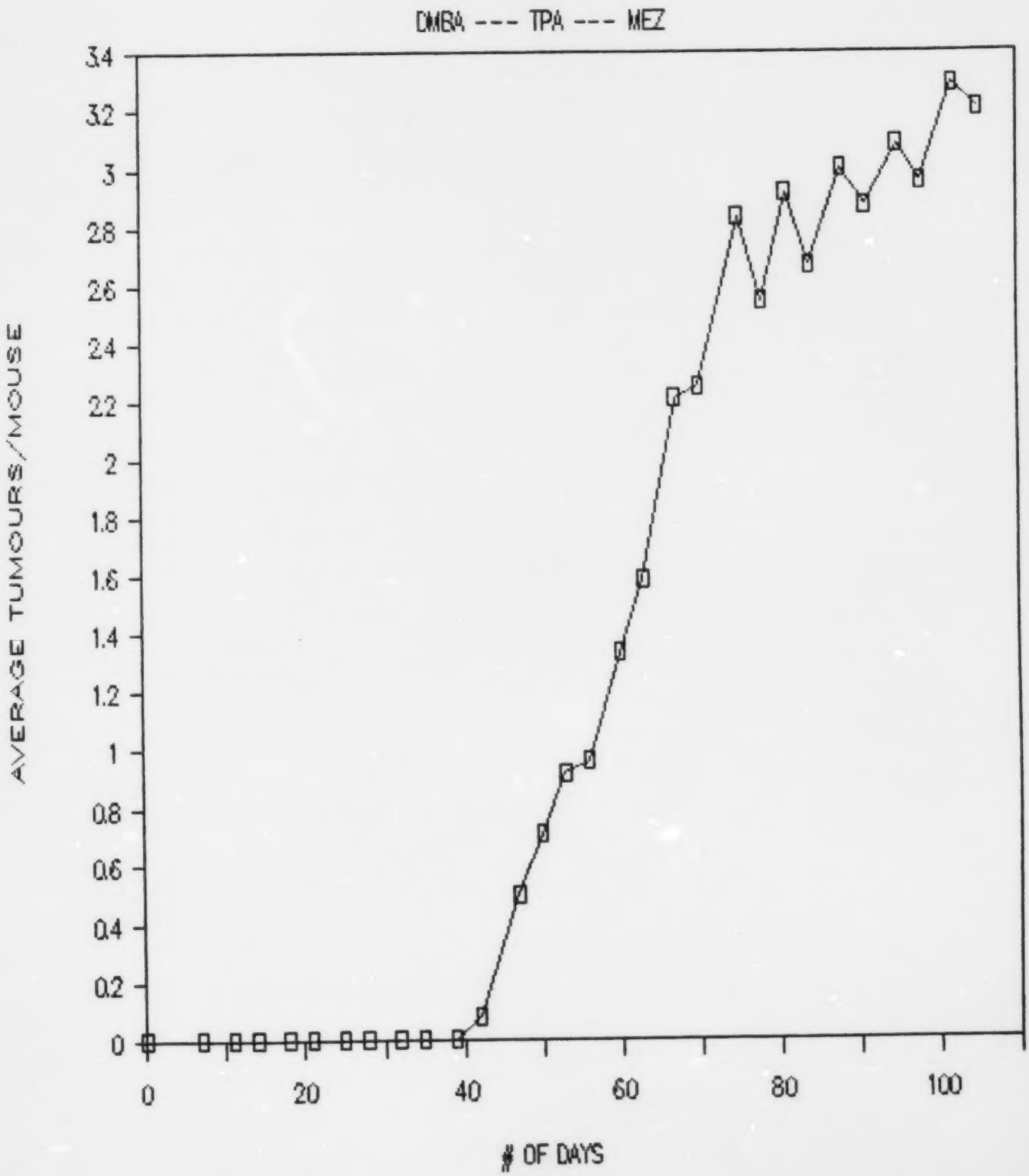
TASK 2 CONTROL GROUP a



TASK 2 CONTROL GROUP b



TASK 3 CONTROL GROUP b



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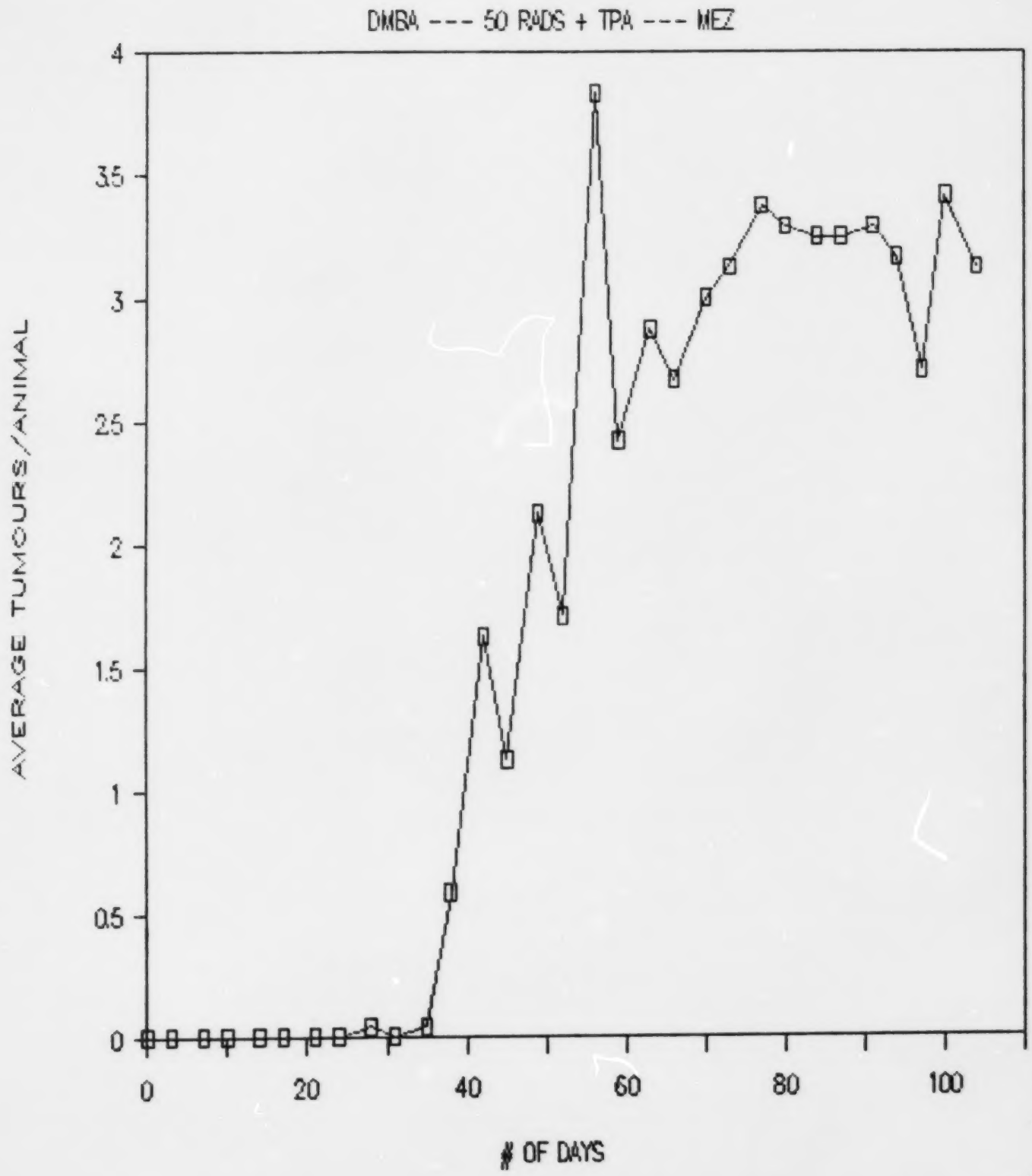

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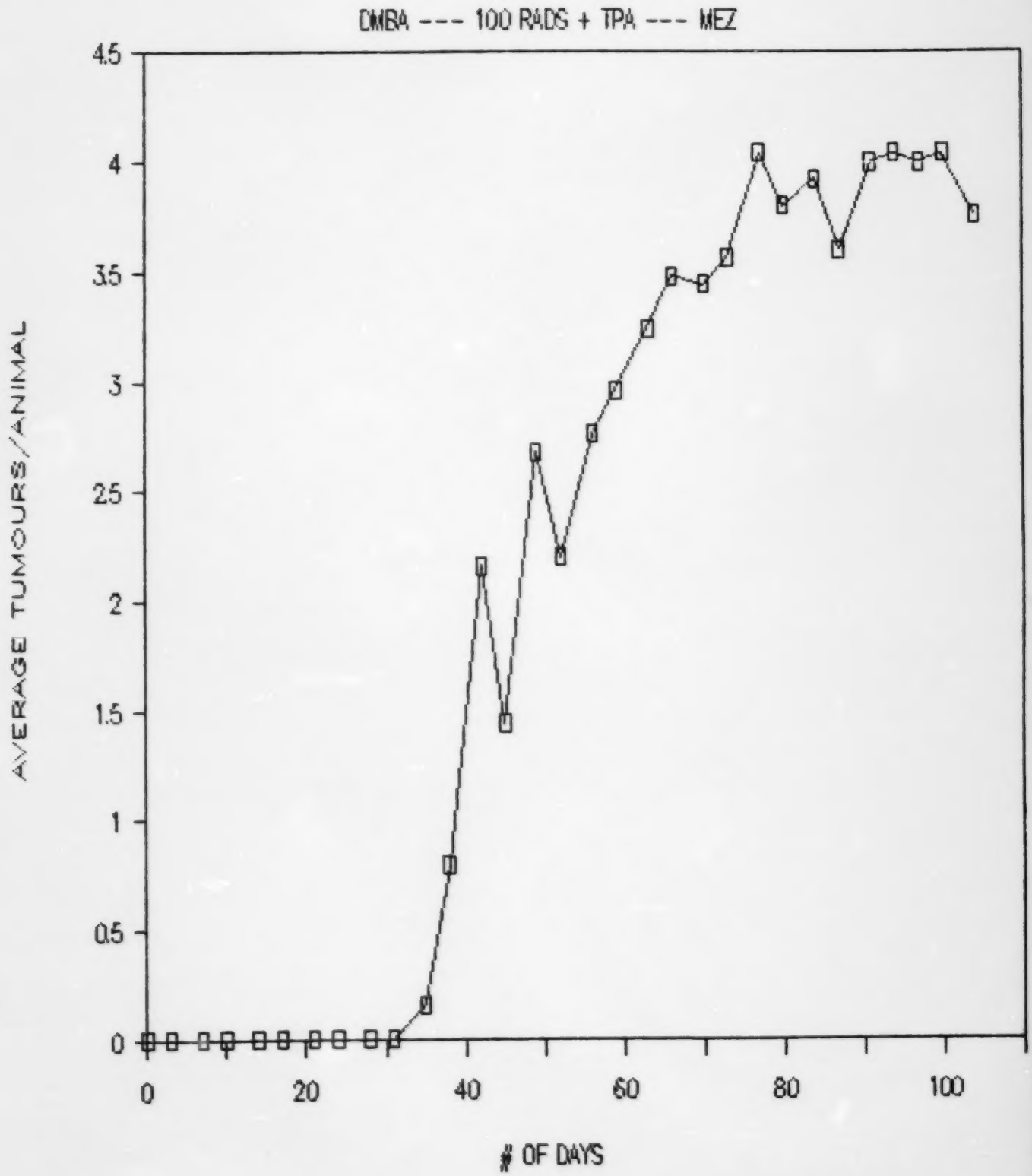

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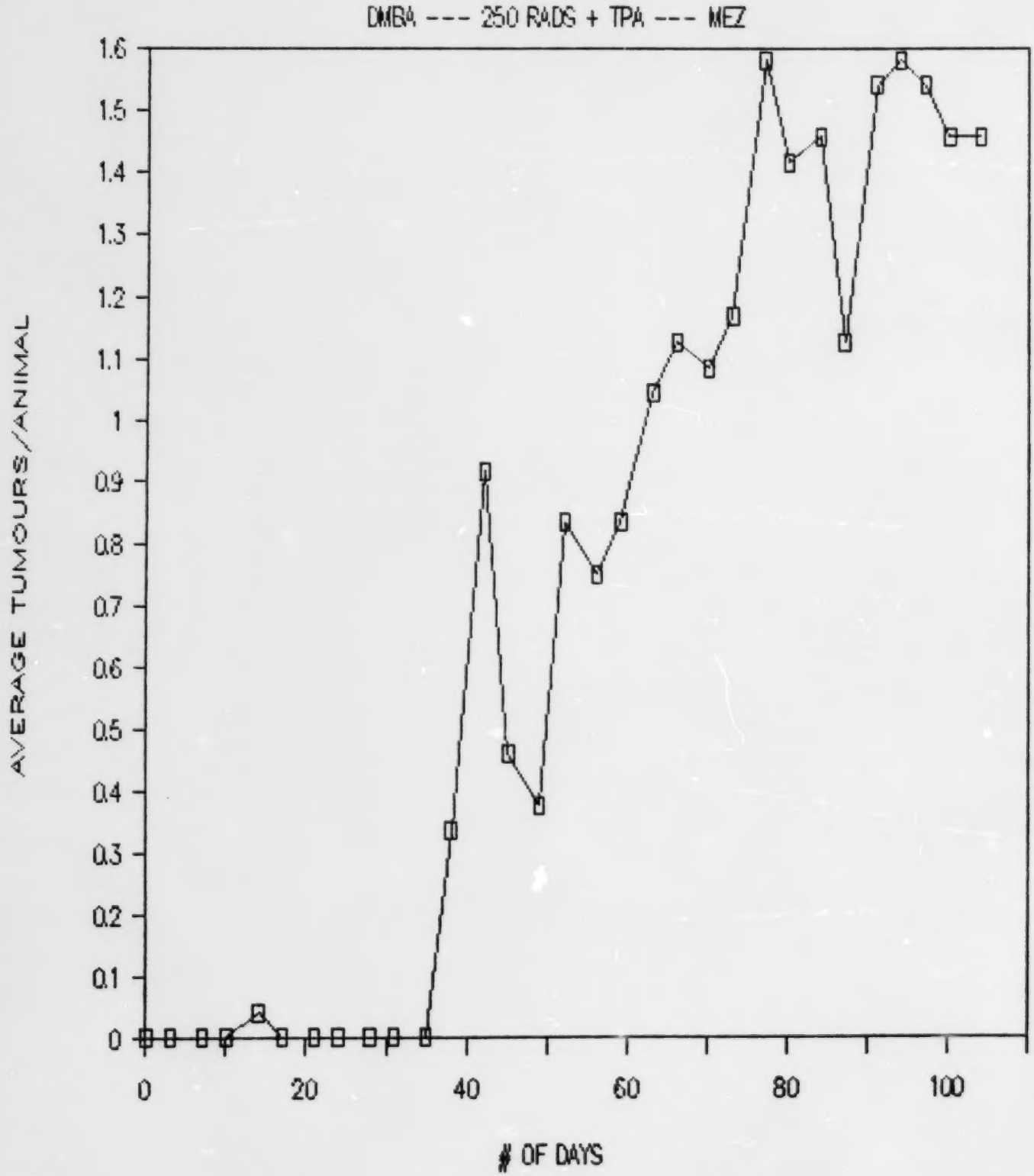
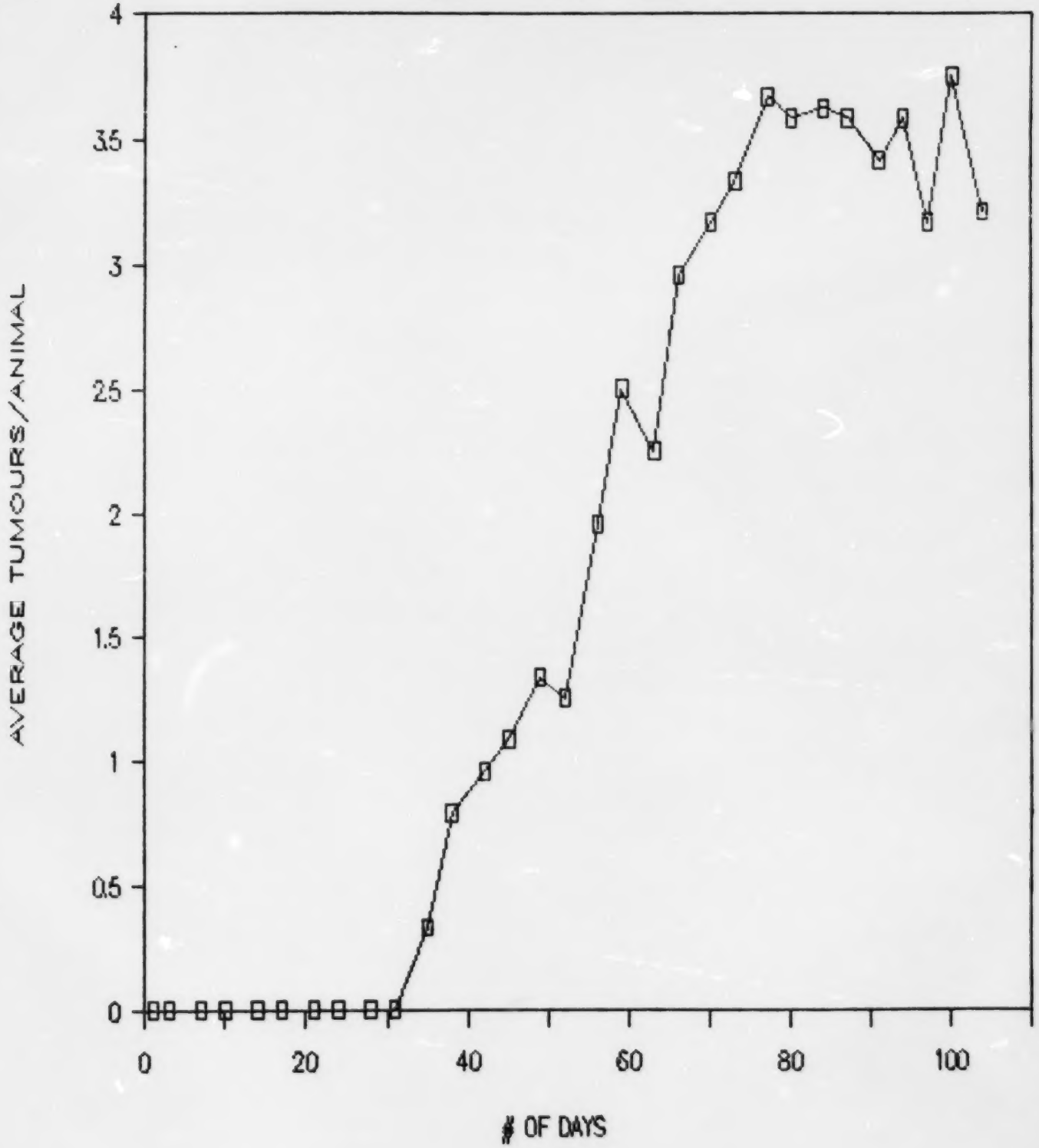
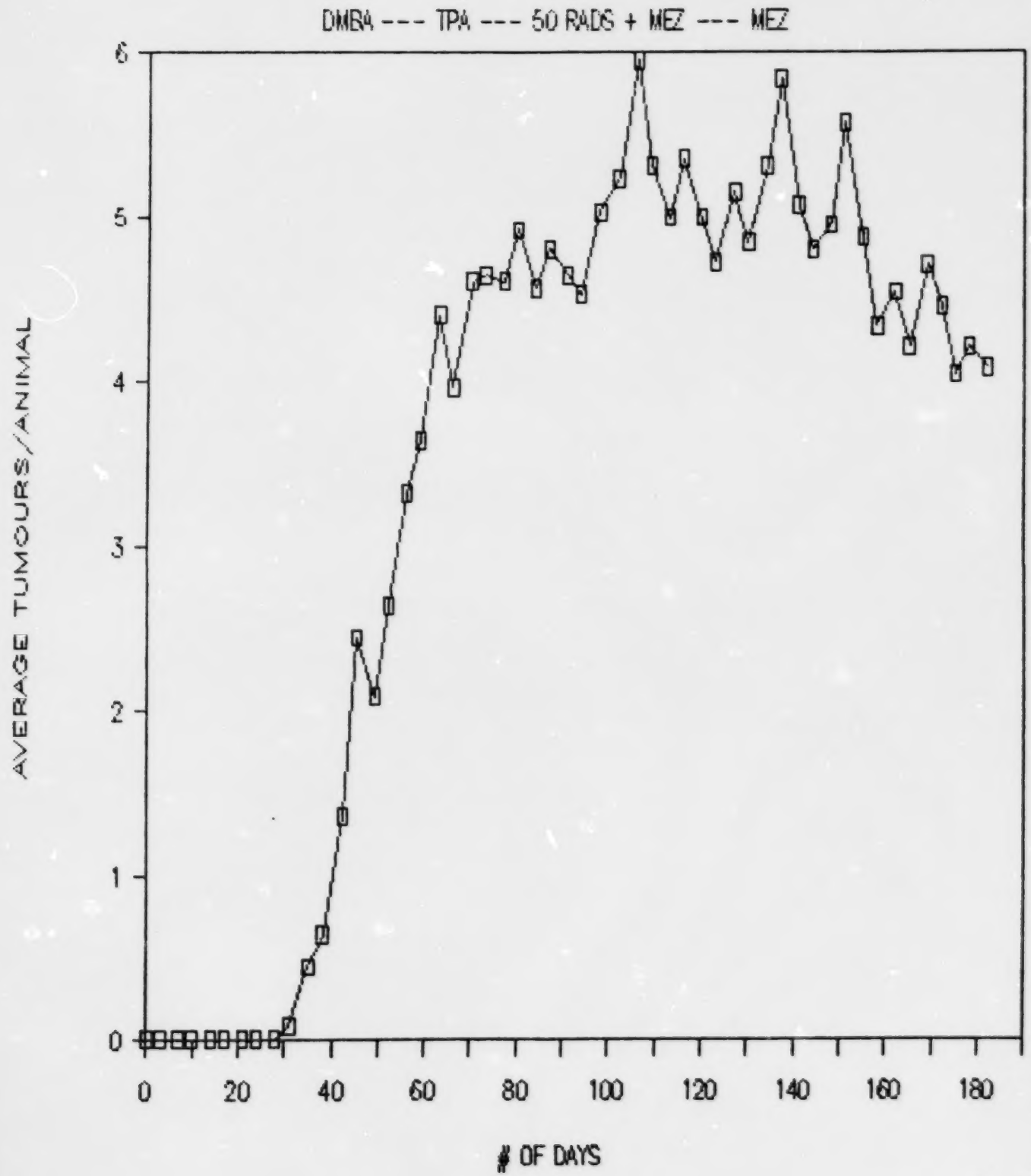


TASK 4 CONTROL GROUP

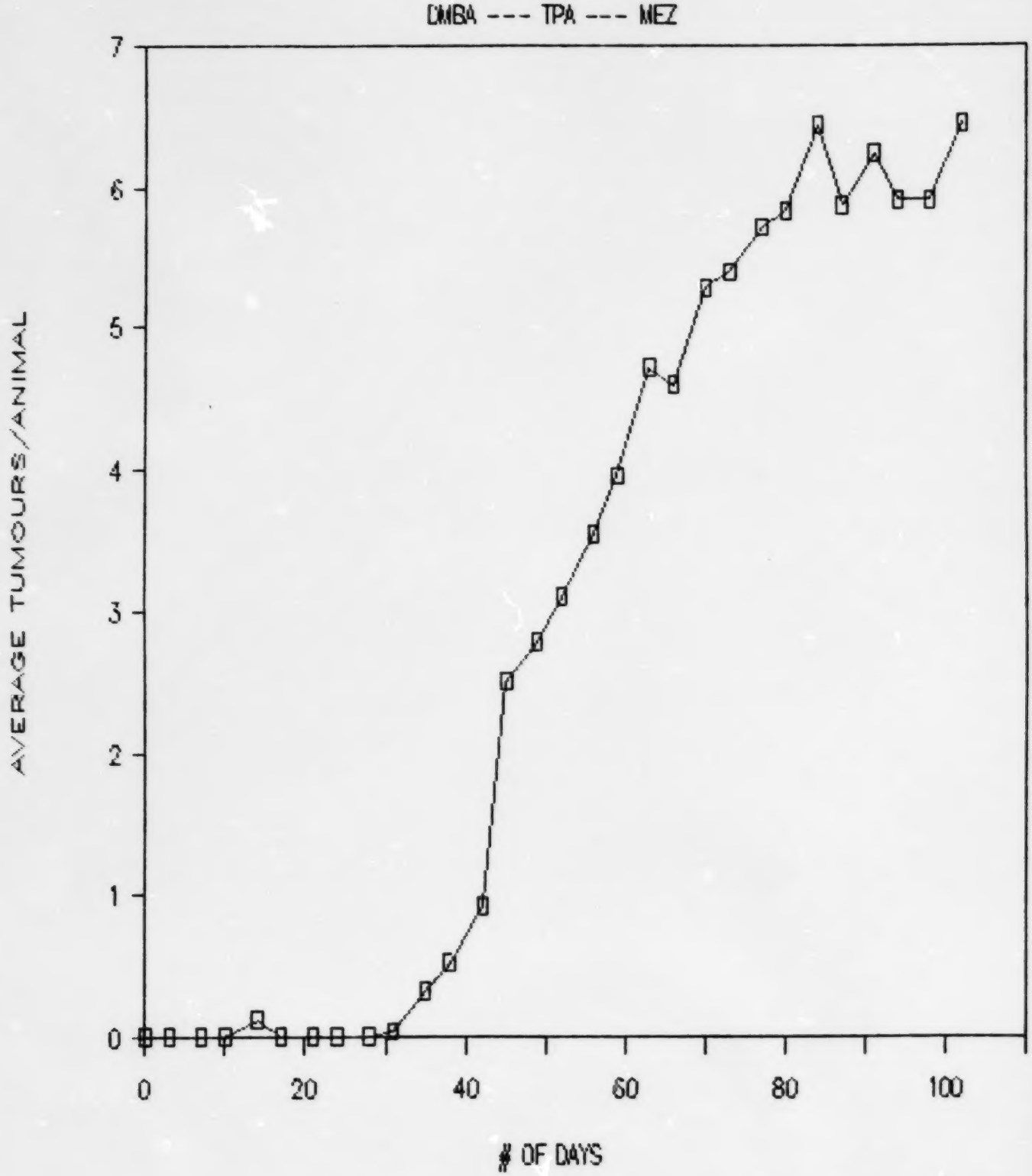
DMBA --- TPA --- MEZ



TASK 5 TEST GROUP



TASK 5 CONTROL GROUP



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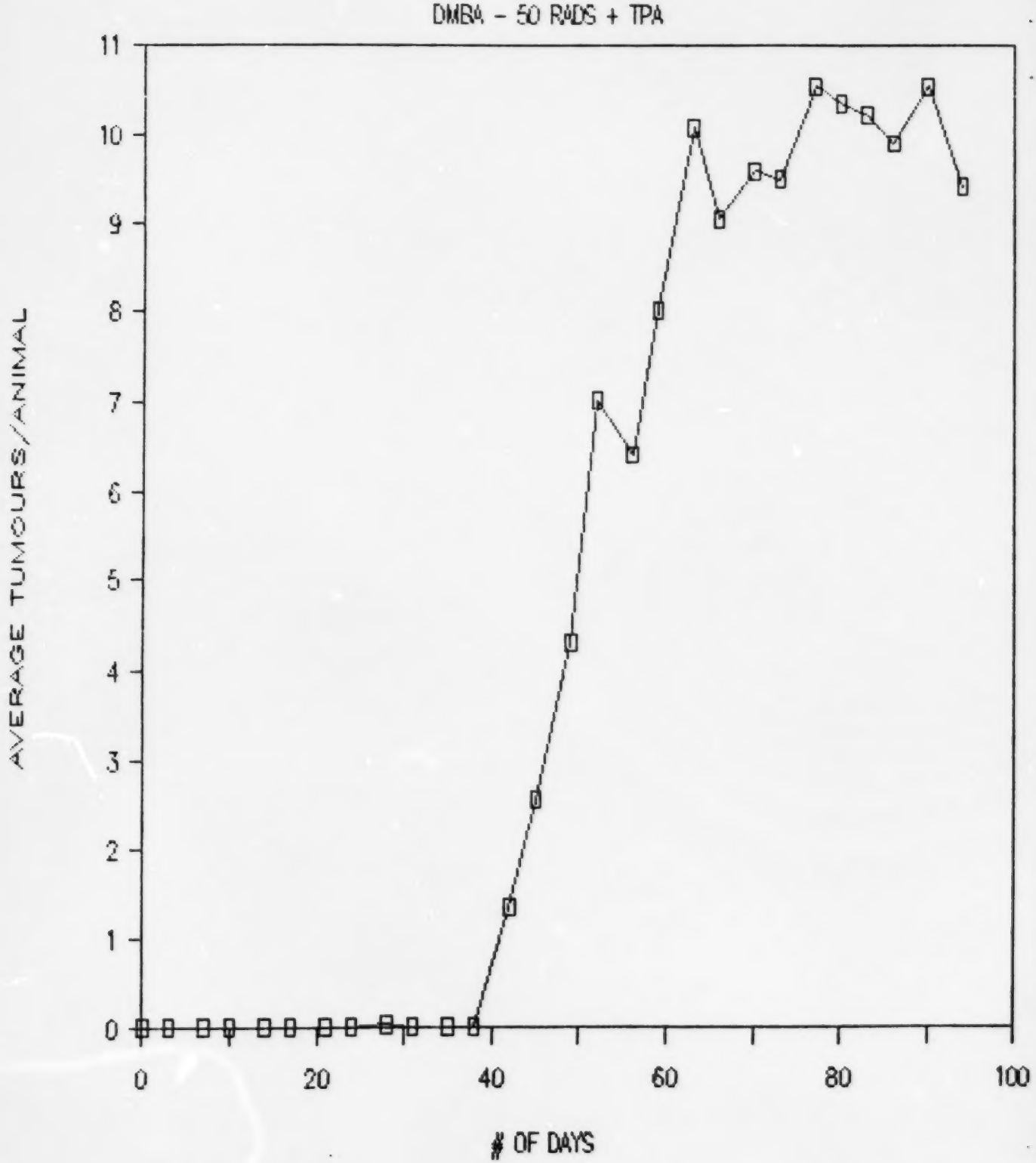
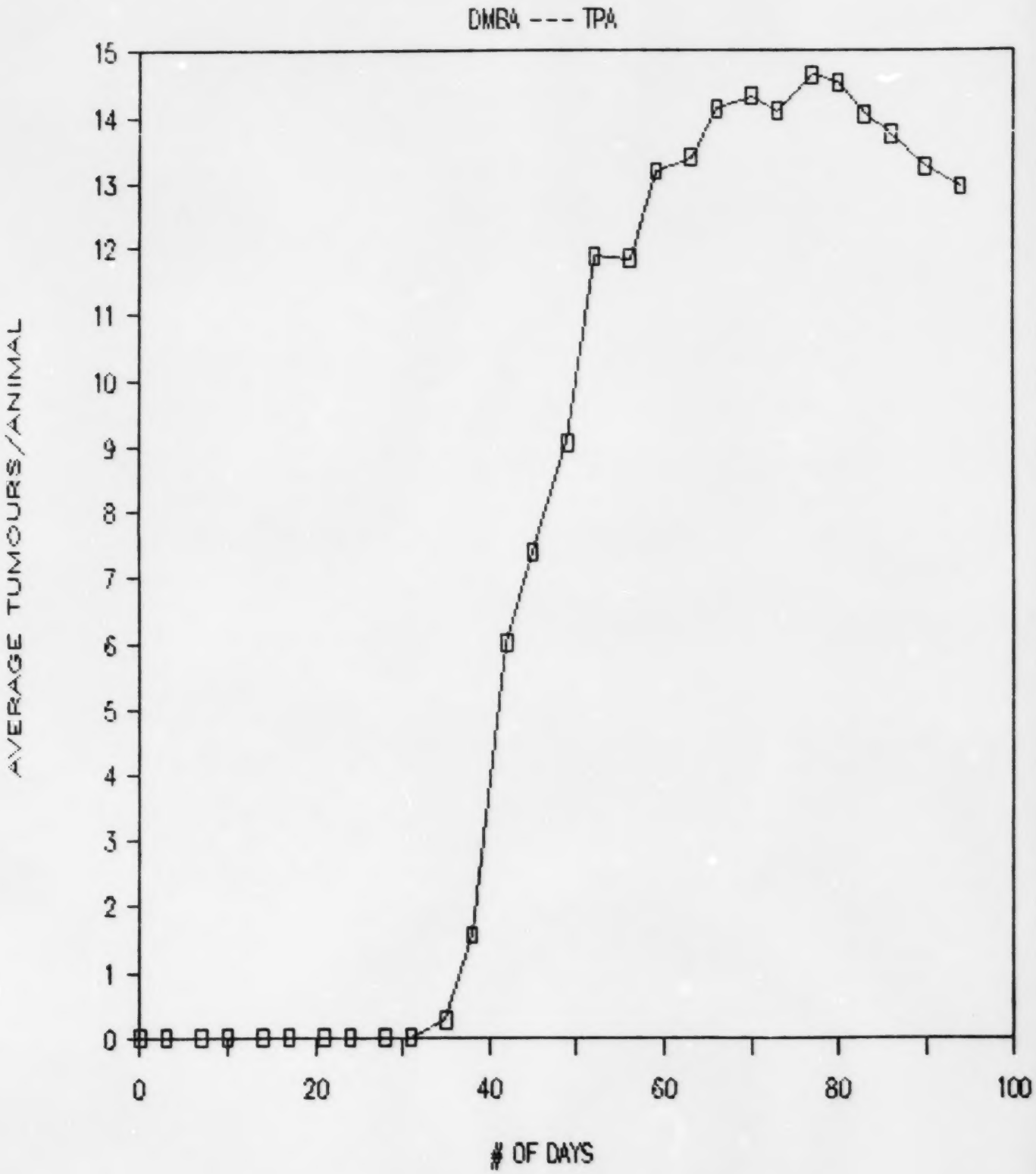
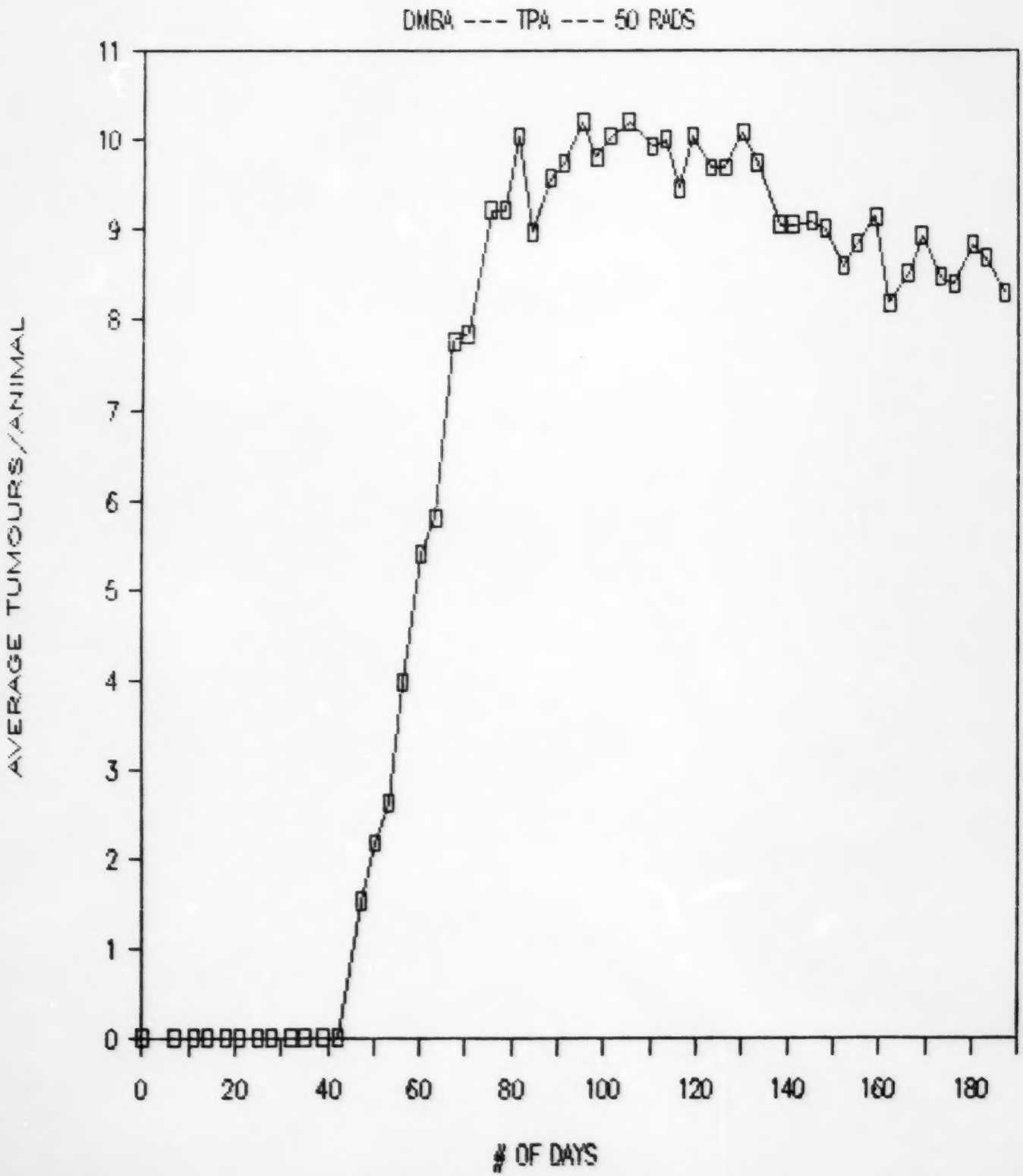


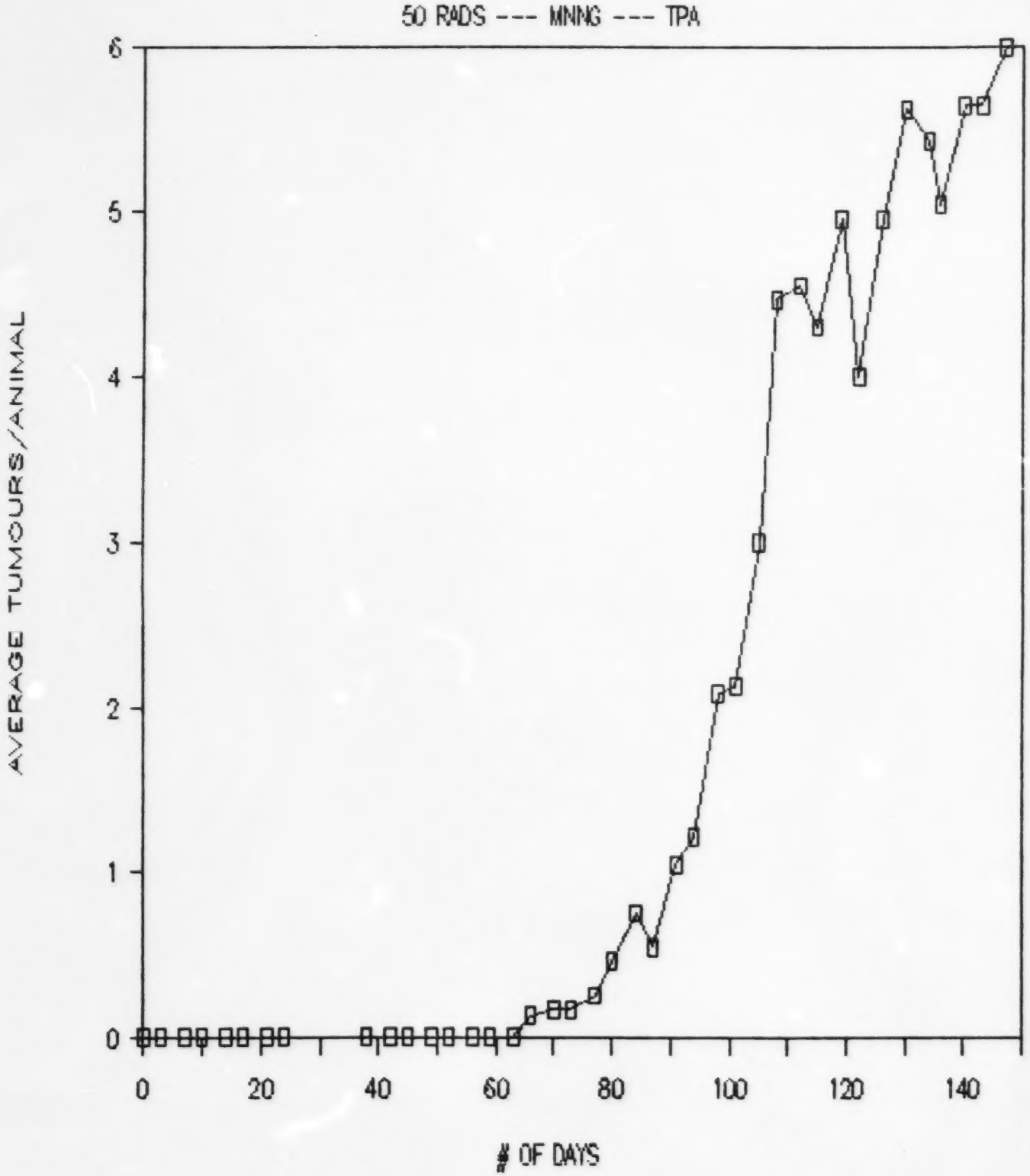
TASK 6 CONTROL GROUP



TASK 7 TEST GROUP



TASK 8 TEST GROUP 1



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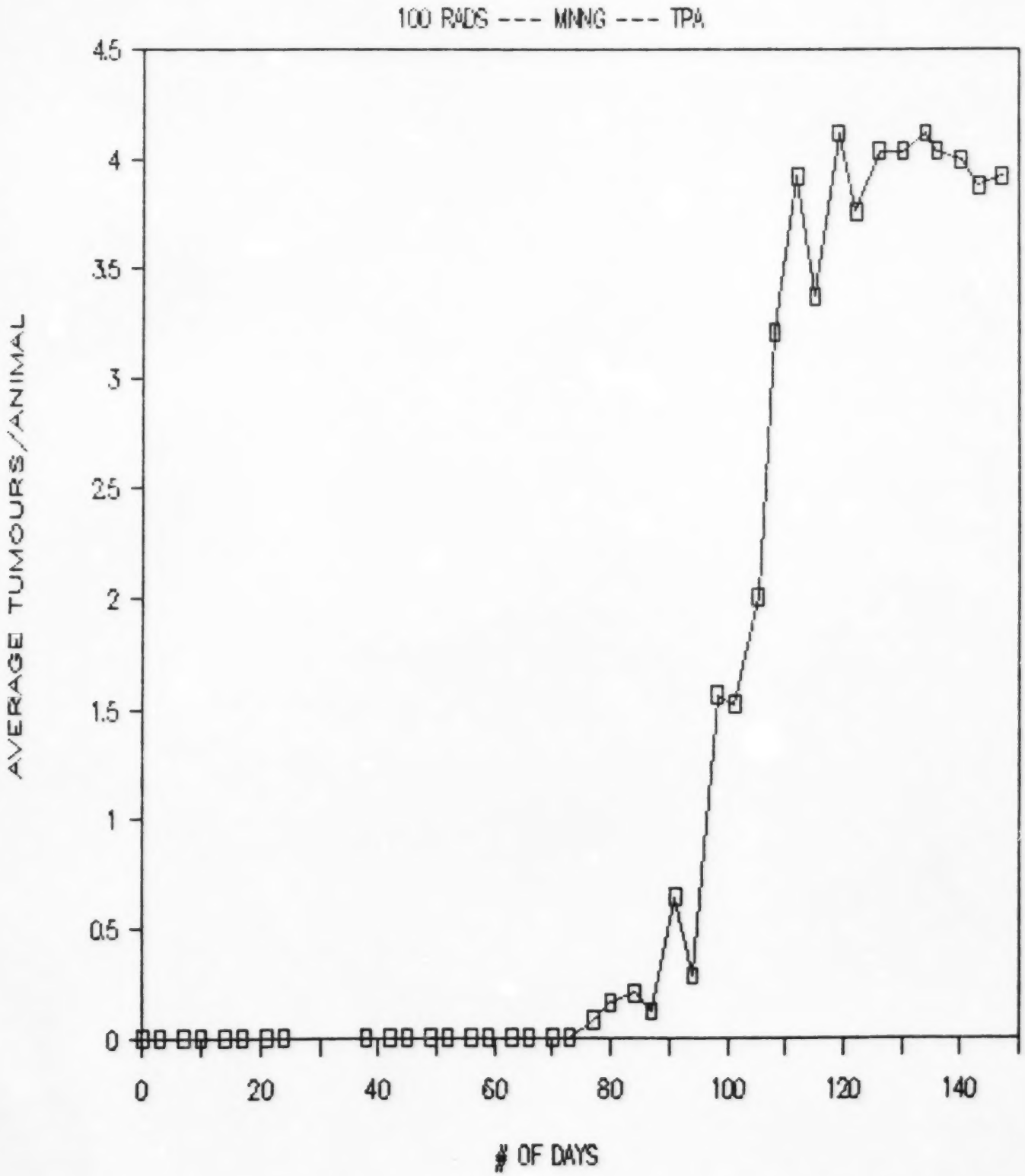
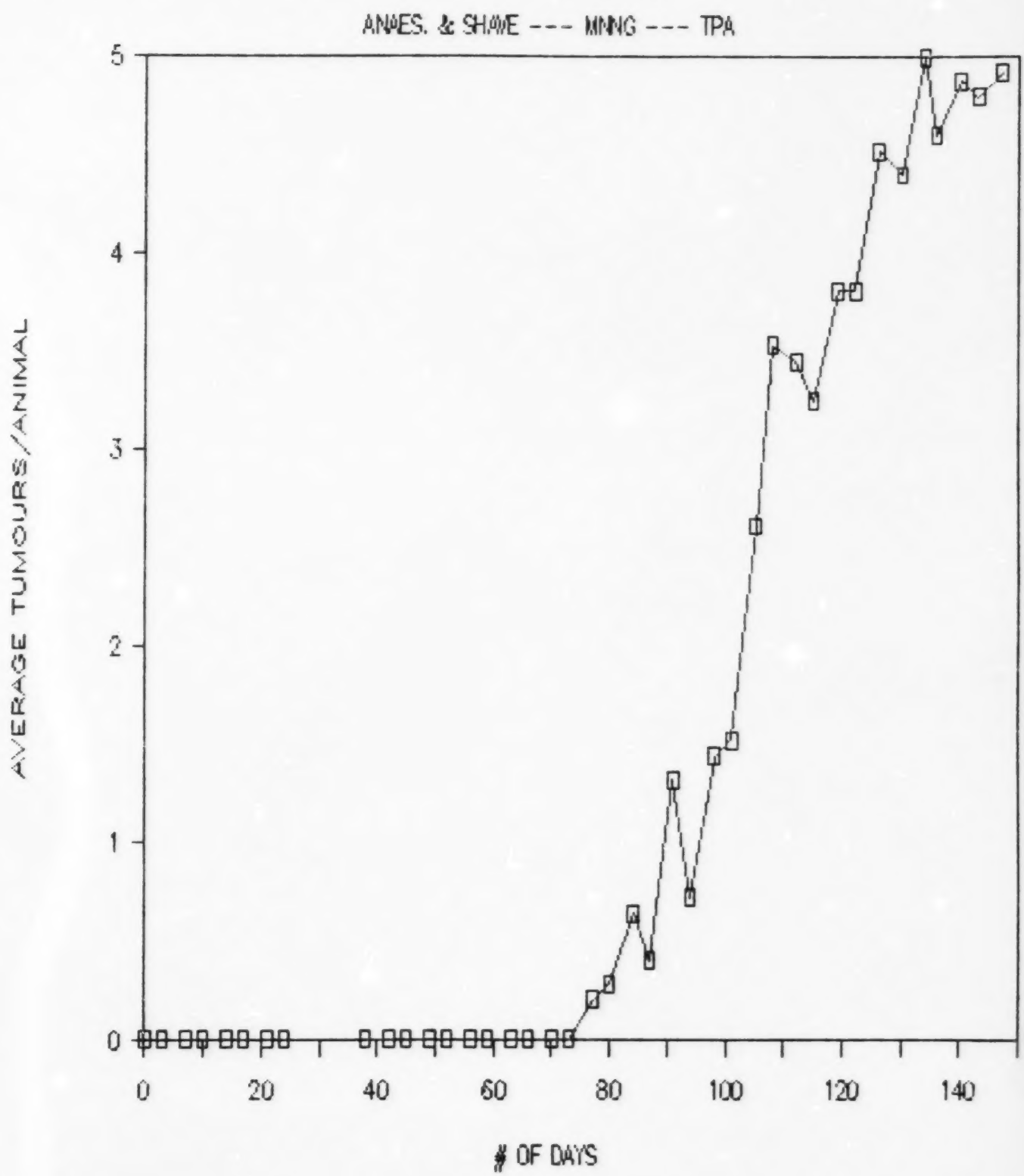
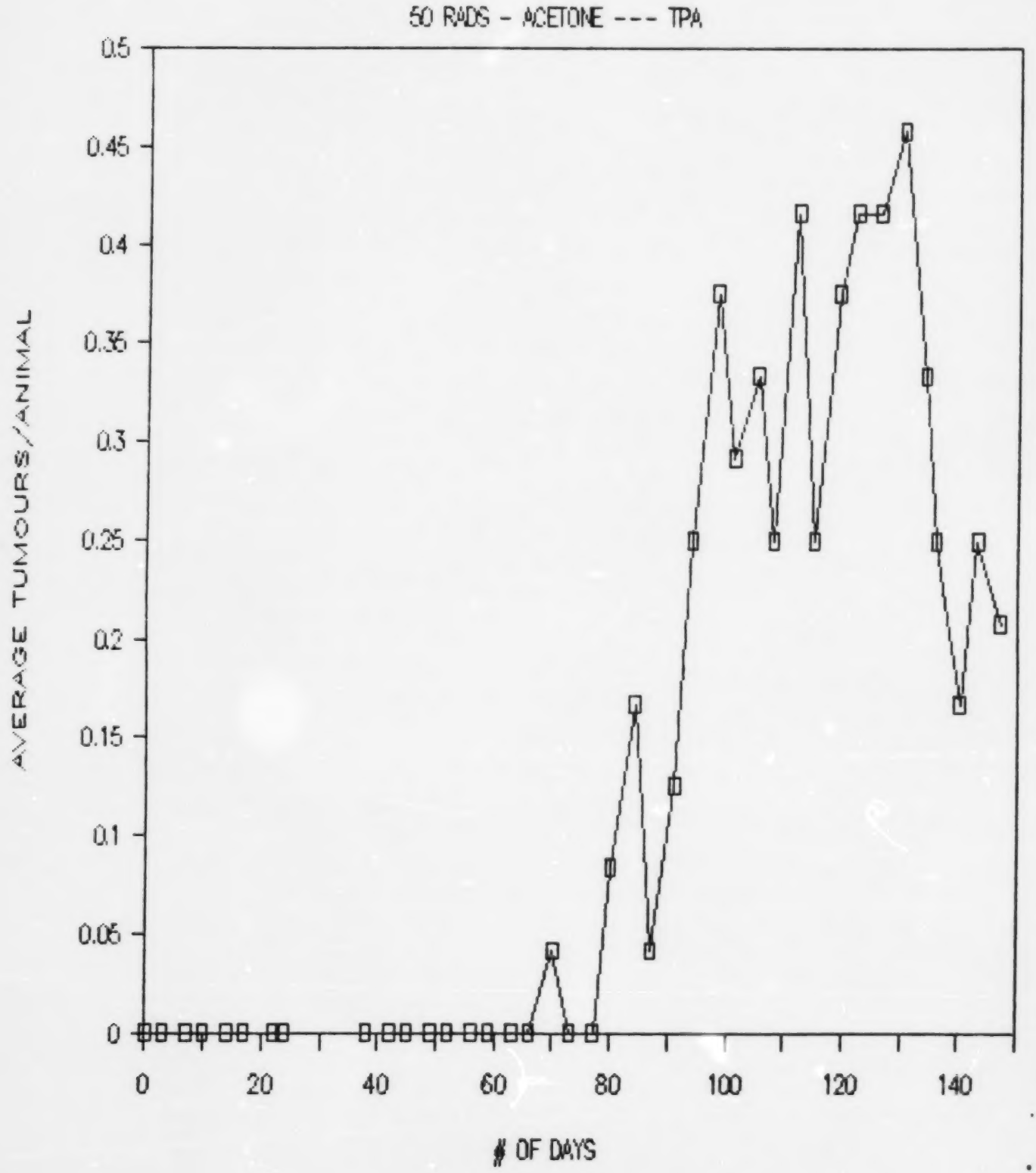


TASK 8 CONTROL GROUP a



TASK 8 CONTROL GROUP b



TASK 8 CONTROL GROUP c